REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
29-04-2016	Final Report	1-Feb-2015 - 31-Jan-2016

4. TITLE AND SUBTITLE
Final Report: UNC Pembroke Laser Scanning Confocal Microscopy Facility

5a. CONTRACT NUMBER
W911NF-15-1-0053

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
106012

6. AUTHORS
Ben A. Bahr

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of North Carolina at Pembroke
One University Drive
P.O. Box 1510
Pembroke, NC 28372 -1510

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
66336-LS-REP.8

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
UNC-Pembroke received funds from the Department of Defense Research and Education Program for Historically Black Colleges and Universities and Minority-Serving Institutions (Equipment/Instrumentation) in order to acquire a Nikon C2+ Confocal Microscopy System. The system strengthens research at UNC-Pembroke, by enhancing the University's Sample Preparation and Microscopy Facility with the capability of laser scanning confocal microscopy. The resulting system has the imaging and resolution capabilities needed to effectively analyze early cellular and cytoskeletal damage, as well as associated synaptic deterioration. The microscope was updated with the

15. SUBJECT TERMS
confocal microscopy, blast-induced TBI, brain imaging

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Ben Bahr
UU	UU	UU	UU		19b. TELEPHONE NUMBER 910-775-4383

Report Title

Final Report: UNC Pembroke Laser Scanning Confocal Microscopy Facility

ABSTRACT

UNC-Pembroke received funds from the Department of Defense Research and Education Program for Historically Black Colleges and Universities and Minority-Serving Institutions (Equipment/Instrumentation) in order to acquire a Nikon C2+ Confocal Microscopy System. The system strengthens research at UNC-Pembroke, by enhancing the University's Sample Preparation and Microscopy Facility with the capability of laser scanning confocal microscopy. The resulting system has the imaging and resolution capabilities needed to effectively analyze early cellular and cytoskeletal damage, as well as associated synaptic deterioration. The microscope was updated with the Nikon Perfect Focus component to allow live cell/intracellular organelle imaging for long-term resolution of cellular changes in different disease states. The funds were also used for 1) transgenic mice housing systems to accommodate mouse strains for addressing experimental objectives in Army projects (RDX blasts, paraoxon exposure), 2) a state-of-the-art immunoblot scanning device in order to compare to histology images and to maximize data obtained from small groups of blast and toxin-treated samples, and 3) a guillotine and freezer for tissue preparation and storage. The Facility supports undergraduate students to engage in cutting edge research and acquire the skills and experience to prepare them for graduate programs and research careers in DOD-relevant areas.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
04/29/2016	7.00	Dario I. Carrasco, Ben A. Bahr, Kevin L. Seburn, Martin J. Pinter. Abnormal response of distal Schwann cells to denervation in a mouse model of motor neuron disease, Experimental Neurology, (04 2016): 0. doi: 10.1016/j.expneurol.2016.02.002
04/29/2016	6.00	Francesca Maltecca, Elisa Baseggio, Francesco Consolato, Davide Mazza, Paola Podini, Samuel M. Young Jr., Ilaria Drago, Ben A. Bahr,, Aldamaria Puliti, Franca Codazzi, Angelo Quattrini, Giorgio Casari. Purkinje neuron Ca2+ influx reduction rescues ataxia in the spinocerebellar ataxia type 28 (SCA28) model, Journal of Clinical Investigation, (01 2015): 263. doi:
TOTAL:		**2**

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received		Paper
TOTAL:		

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Bahr BA. Materials and compound combinations for cathepsin B enhancement and methods of use for treating Alzheimer's disease, mild cognitive impairment (MCI), dementia, forms of ? synucleinopathy, traumatic brain injury, cardiomyopathy, eye disease, and skin damage (U.S. Provisional Patent Application No. 62/262,848- filed 12/3/15; 204 claims); assigned to UNC–Pembroke

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Marquitta Smith	0.00
Karen Farizatto	0.00
FTE Equivalent:	**0.00**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Ben A. Bahr	0.00	
FTE Equivalent:	**0.00**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME
Cecily Ivey
Total Number: **1**

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Heather Romine	0.00
FTE Equivalent:	**0.00**
Total Number:	**1**

Sub Contractors (DD882)

Inventions (DD882)

5 **Materials and compound combinations for cathepsin B enhancement and methods of use for treating Alzheimer's disease,**

Patent Filed in US? (5d-1) Y

Patent Filed in Foreign Countries? (5d-2) N

Was the assignment forwarded to the contracting officer? (5e) N

Foreign Countries of application (5g-2):

5a: Ben A. Bahr

5f-1a: UNC Pembroke

5f-c: 1 University Dr.

Pembroke NC 28372

Scientific Progress

Scientific progress and accomplishments

1. Foreword

UNC-Pembroke received funds from the Department of Defense Research and Education Program for Historically Black Colleges and Universities and Minority-Serving Institutions (Equipment/Instrumentation) in order to acquire a Confocal Microscopy System. The system was also upgraded with Nikon's Perfect Focus option with motorized diascopic detector, resulting in enhanced capabilities with long-term high-power resolution necessary for brain samples and labeling probes. The facility in place today now has the imaging and resolution capabilities needed to effectively analyze early signs of cellular and cytoskeletal damage, as well as associated synaptic deterioration and astroglial activation events.

2. Statement of the Problem

State-of-the-art confocal microscopy was needed to strengthen the biomedical research at UNC-Pembroke, by enhancing the University's existing Sample Preparation and Microscopy Facility with the capability of laser scanning confocal microscopy and image capture. The previously existing microscopy facility did not have the imaging and resolution capabilities needed to effectively analyze distinct cellular and synaptic damage events during different disease states. The requested equipment included the Nikon C2+ Confocal Microscopy System. The system was needed to support the biomedical, Army-relevant neuroscience-focused research that is actively being conducted at UNC-Pembroke's Biotechnology Research and Training Center (BRTC). The Nikon system was needed in order to provide the significantly greater image resolution and magnification needed to support Dr. Ben Bahr's neuroscience research in the areas of traumatic brain damage, excitotoxic vulnerability, and Alzheimer's disease. The system will provide significantly improved resolution of data images and biological samples, which is needed to understand cellular and synaptic changes associated with blast-induced neurodegeneration and in different disease states.

This Nikon system was requested since Nikon has the reputation as a world leader in advanced biological imaging, and their confocal system can work in tandem with the existing Nikon AZ100 microscope for macro images of large brain regions. We requested the C2 model since it provides both advanced imaging quality, functionality, flexibility for growth, and a reasonable cost. We also requested the Nikon product since a local sales and support team is available in North Carolina (RTP) which will provide sales, service, training, and support in a timely fashion as they did for the AZ100 microscope and software.

3. Summary of the most important results

The confocal microscopy equipment was installed by experts from Nikon, and the required training for optimal use of the system was provided to several members of the Bahr Lab as well as a few other professors from other disciplines. The confocal system and facility has supported Army-relevant neuroscience research in the Bahr Lab. Dr. Bahr's research aims to understand the pathogenic cascade of events that initiates from excitotoxic insults (e.g. trauma, stroke, seizures) and leads to the deterioration of brain structures and functions.

BLAST-INDUCED NEURODEGENERATION

Particularly important to the Army, the growing number of traumatic brain injury (TBI) cases associated with military service warrants Dr. Bahr's research on blast-induced neurodegeneration. Explosives create shockwaves that cause blast-induced neurotrauma, one of the most common types of TBI linked to military service. Survivable blast-induced TBIs are often associated with reduced cognitive and behavioral functions due to a variety of factors. The confocal system has been assisting a study of the direct effects of explosive blasts on brain tissue. We removed systemic factors by utilizing rat hippocampal slices maintained in culture. The long-term slice cultures were briefly sealed air-tight in serum-free medium (SFM), lowered into a 37° C water-filled blast chamber, and 1.7-gram assemblies of cyclotrimethylene trinitramine (RDX) were detonated 15 cm outside the chamber, creating a distinct shockwave recorded at the culture plate position. Compared to control mock treatment groups of slices that received equal SFM submerge time, the blast impacts caused a dose-dependent reduction in the AMPA receptor subunit GluR1. Only a small reduction was found in slices exposed to a single RDX blast, harvested 1-2 days post-blast. However, hippocampal slices that received two consecutive RDX blasts 4 min apart exhibited a 26-50 % reduction in GluR1, and it was further reduced by 60-67% after three consecutive blasts. The presynaptic markers synaptophysin and synaptotagmin were found to have similar susceptibility to multiple blast exposures as the postsynaptic protein. With confocal microscopy, the synaptic marker staining in distinct patterns around pyramidal neurons was greatly disrupted by the blast trauma. In the slice samples with clear indications of blast-induced synaptic compromise, the level of blasts used did not produce evidence of astroglial activation as measures of GFAP did not increase (a small decrease was in fact found). Actin levels were unchanged and Fluoro-Jade staining found no indication of degenerating neurons in slice cultures exposed to three RDX blasts, suggesting that synaptic alterations can occur in the absence of cellular degeneration and gliosis. Together, these results indicate that detonated RDX explosives cause distinct losses of synaptic proteins before cell death, perhaps explaining the cognitive deficits in those blast-induced TBIs with no detectable neuropathology.

PROTEIN ACCUMULATION DISEASES

Dr. Bahr's research also aims to understand how age-related protein accumulation stress can be reduced for therapeutic purposes. Distinct protein accumulation events are suspected to lead to Alzheimer's, Parkinson's, Huntington's, ALS, and other diseases. The new confocal microscopy system assisted the following study being prepared for publication:

Accumulating protein species can lead to the activation of proteasomal and lysosomal pathways. However, many studies have indicated that the two pathways exhibit stress during Alzheimer-type protein accumulation events. In particular, the Aβ42 peptide has been shown to influence proteasomes and overall proteostasis. Here, low concentration Aβ42 applied to rat hippocampal slice cultures was found to reduce proteasome activity in correspondence with increased tau phosphorylation, as well as cause a significant loss of synaptophysin, a sensitive marker of synaptic integrity. When the slice cultures were treated with the proteasome inhibitor lactacystin, the nearly complete and rapid reduction in proteasome activity was not associated with lysosomal compromise, but rather with a >50% increase in activity of the lysosomal enzyme cathepsin B (CatB). Interestingly, the potential compensatory CatB response increased further over additional days of lactacystin treatment. To further assess this apparent inverse relationship between the proteasomal and lysosomal pathways, we tested whether enhancing the active form of CatB leads to proteasomal attenuation. Using the CatB-enhancing agent Z-Phe-Ala-diazomethylketone (PADK), an inverse effect was not found, but rather what appears to be an opposite effect, i.e. a tendency to increase proteasome activity. Surprisingly, in hippocampal slices with Aβ42-mediated proteasomal compromise, PADK indeed increased the proteasome activity to levels comparable to those found in control slices. Furthermore, PADK also reduced Aβ42-mediated tau phosphorylation, an event recently implicated as a consequence of changes in protein clearance efficiency. Such efficiency may involve cross-talk between proteasomes and lysosomes. These results suggest a distinct interaction between proteasomal and lysosomal systems, and they point to potential dual modulation against protein accumulation pathology linked to Alzheimer's disease and other dementias. New activity probes are currently being tested to assess modulation of protein clearance pathways in living brain tissue cultures.

INVASIVE FIRE ANTS

Professor Lisa Kelly of UNC Pembroke has been trained on the new confocal system. Dr. Kelly's research interest in the trophic ecology of the invasive fire ant has begun to benefit from the wide field view and long working distances of a confocal imaging system. The macro-observations of individual ants have already achieved images never seen with such resolution of ant body morphology. This will allow for diagnostic identification and may allow the capture of subtle differences in morphology of monogyne and polygyne fire ants --- differences that may be unapparent using conventional stereoscopic microscopes. The confocal system has provided a unique opportunity for undergraduate student experience and training.

NEMATODE INTERACTIONS

Professor Len Holmes of UNC Pembroke and his staff are being trained on the new confocal system. His research interest includes the interaction of a nematode species (H. bacteriophora) with its bacterial symbiotic partner (P. luminescens). The addition of the confocal microscope system will greatly facilitate Professor Holmes' work on the nematode-bacteria interactions, using novel fluorescent probes and image analysis.

NEUROMUSCULAR JUNCTION MODEL

Professor Robert Poage of UNC Pembroke is being trained on the new confocal system. Dr. Poage's research interest involves the dynamics of structural networks that modulate the neuromuscular junction and its functionality. This neuromuscular junction model, which uses frogs and rodents, has been among the most widely studied synaptic models. Dr. Poage's work manipulates ultrastructural features of the neuromuscular junction using neurotoxins, genetic regulation, or ionic manipulations. With the new microscope system, the Poage Lab will be able to probe important questions regarding the presynaptic calcium channel localization.

RESEARCH TRAINING CAPACITY

The new equipment has strengthened the research training capacity of UNCP. UNCP is a minority serving institution with 16% of the students being Native American, 32% are African American, and 4% are Hispanic/Latino. Faculty are dedicated to meeting the academic needs of minority students including mentoring undergraduate students in research and advanced technologies like confocal microscopy. Dr. Bahr's group has exposed many students to confocal techniques performed with the confocal imaging system, including undergraduates, a few Master students, as well as visiting and shadowing high school students. With the new system, UNCP students are engage in cutting edge research and are able acquire the skills and experience to prepare them for graduate programs and research careers in DOD-relevant areas. In addition, the STEM faculty of UNCP are committed to working with local K-12 students and teachers and local community college students. These students are regularly invited to tour and work in the Bahr Lab and other STEM labs on campus.

4. Bibliography (including previous studies that used confocal imaging)

Bahr BA, Wisniewski ML, and Butler D (2012) Positive lysosomal modulation as a unique strategy to treat age-related protein accumulation diseases. Rejuvenation Res 15:189-197.
Bendiske J, Caba E, Brown QB, and Bahr BA (2002) Intracellular deposition, microtubule destabilization, and transport failure: An 'early' pathogenic cascade leading to synaptic decline. J Neuropathol Exp Neurol 61:640-650.
Butler D, Brown QB, Chin D, Batey L, Karim S, Mutneja MS, Karanian DA, and Bahr BA (2005) Cellular responses to protein accumulation involve autophagy and lysosomal enzyme activation. Rejuvenation Res 8:227-237.
Butler D, Hwang J, Estick C, Nishiyama A, Kumar SS, Baveghems C, Young-Oxendine HB, Wisniewski ML, Charalambides A, and Bahr BA (2011) Protective effects of positive lysosomal modulation in Alzheimer's disease transgenic mouse models. PLoS One 6: e20501.
Carrasco DI, Bahr BA, Seburn KL, and Pinter MJ (2016) Abnormal response of distal Schwann cells to denervation in a mouse model of motor neuron disease. Exp Neurol 278:116-126.
Farizatto KL, Ikonne US, Ferrari MF, and Bahr BA (2016) Interaction between proteasomal and lysosomal systems influences Aβ effects in hippocampus. In preparation.
Filipovic R, Kumar SS, Bahr BA, and Loturco J (2014) Slice culture method for studying migration of neuronal progenitor cells derived from human embryonic stem cells (hESC). Curr Protoc Stem Cell Biol 29:1H.7.1-1H.7.14.
Hoffmann DB, Williams SK, Bojcevski J, Müller A, Stadelmann C, Naidoo V, Bahr BA, Diem R, and Fairless R (2013) Calcium influx and calpain activation mediate preclinical retinal neurodegeneration in autoimmune optic neuritis. J Neuropathol Exp Neurol 72:745-757.
Maltecca F, Baseggio E, Consolato F, Mazza D, Podini P, Young SM Jr, Drago I, Bahr BA, Puliti A, Codazzi F, Quattrini A, and Casari G (2015) Purkinje neuron Ca2+ influx reduction rescues ataxia in the spinocerebellar ataxia type 28 (SCA28) model. J Clin Invest 125:263-274.
Melo CV, Okumoto S, Gomes JR, Baptista MS, Bahr BA, Frommer WB, and Duarte CB (2013) Spatiotemporal resolution of BDNF neuroprotection against glutamate excitotoxicity in cultured hippocampal neurons. Neuroscience 237:66-86.
Munirathinam S and Bahr BA (2004). Repeated contact with subtoxic soman leads to synaptic vulnerability in hippocampus. J Neuroscience Res 77:739-746.
Smith M, Farizatto KL, Piehler T, Benjamin R, Almeida MF, and Bahr BA (2016) Blast waves from detonated RDX explosive lead to synaptic protein loss in hippocampal slice cultures.
Wisniewski ML, Hwang J, and Bahr BA (2011) Submicromolar Aβ42 reduces hippocampal glutamate receptors and presynaptic markers in an aggregation-dependent manner. Biochim Biophys Acta (Mol. Basis of Disease) 1812:1664-1674.

Technology Transfer

Scientific progress and accomplishments

1. Foreword

UNC-Pembroke received funds from the Department of Defense Research and Education Program for Historically Black Colleges and Universities and Minority-Serving Institutions (Equipment/Instrumentation) in order to acquire a Confocal Microscopy System. The system was also upgraded with Nikon's Perfect Focus option with motorized diascopic detector, resulting in enhanced capabilities with long-term high-power resolution necessary for brain samples and labeling probes. The facility in place today now has the imaging and resolution capabilities needed to effectively analyze early signs of cellular and cytoskeletal damage, as well as associated synaptic deterioration and astroglial activation events.

2. Statement of the Problem

State-of-the-art confocal microscopy was needed to strengthen the biomedical research at UNC-Pembroke, by enhancing the University's existing Sample Preparation and Microscopy Facility with the capability of laser scanning confocal microscopy and image capture. The previously existing microscopy facility did not have the imaging and resolution capabilities needed to effectively analyze distinct cellular and synaptic damage events during different disease states. The requested equipment included the Nikon C2+ Confocal Microscopy System. The system was needed to support the biomedical, Army-relevant neuroscience-focused research that is actively being conducted at UNC-Pembroke's Biotechnology Research and Training Center (BRTC). The Nikon system was needed in order to provide the significantly greater image resolution and magnification needed to support Dr. Ben Bahr's neuroscience research in the areas of traumatic brain damage, excitotoxic vulnerability, and Alzheimer's disease. The system will provide significantly improved resolution of data images and biological samples, which is needed to understand cellular and synaptic changes associated with blast-induced neurodegeneration and in different disease states.

This Nikon system was requested since Nikon has the reputation as a world leader in advanced biological imaging, and their confocal system can work in tandem with the existing Nikon AZ100 microscope for macro images of large brain regions. We requested the C2 model since it provides both advanced imaging quality, functionality, flexibility for growth, and a reasonable cost. We also requested the Nikon product since a local sales and support team is available in North Carolina (RTP) which will provide sales, service, training, and support in a timely fashion as they did for the AZ100 microscope and software.

3. Summary of the most important results

The confocal microscopy equipment was installed by experts from Nikon, and the required training for optimal use of the system was provided to several members of the Bahr Lab as well as a few other professors from other disciplines. The confocal system and facility has supported Army-relevant neuroscience research in the Bahr Lab. Dr. Bahr's research aims to understand the pathogenic cascade of events that initiates from excitotoxic insults (e.g. trauma, stroke, seizures) and leads to the deterioration of brain structures and functions.

BLAST-INDUCED NEURODEGENERATION

Particularly important to the Army, the growing number of traumatic brain injury (TBI) cases associated with military service warrants Dr. Bahr's research on blast-induced neurodegeneration. Explosives create shockwaves that cause blast-induced neurotrauma, one of the most common types of TBI linked to military service. Survivable blast-induced TBIs are often associated with reduced cognitive and behavioral functions due to a variety of factors. The confocal system has been assisting a study of the direct effects of explosive blasts on brain tissue. We removed systemic factors by utilizing rat hippocampal slices maintained in culture. The long-term slice cultures were briefly sealed air-tight in serum-free medium (SFM), lowered into a 37°C water-filled blast chamber, and 1.7-gram assemblies of cyclotrimethylene trinitramine (RDX) were detonated 15 cm outside the chamber, creating a distinct shockwave recorded at the culture plate position. Compared to control mock treatment groups of slices that received equal SFM submerge time, the blast impacts caused a dose-dependent reduction in the AMPA receptor subunit GluR1. Only a small reduction was found in slices exposed to a single RDX blast, harvested 1-2 days post-blast. However, hippocampal slices that received two consecutive RDX blasts 4 min apart exhibited a 26-50 % reduction in GluR1, and it was further reduced by 60-67% after three consecutive blasts. The presynaptic markers synaptophysin and synaptotagmin were found to have similar susceptibility to multiple blast exposures as the postsynaptic protein. With confocal microscopy, the synaptic marker staining in distinct patterns around pyramidal neurons was greatly disrupted by the blast trauma. In the slice samples with clear indications of blast-induced synaptic compromise, the level of blasts used did not produce evidence of astroglial activation as measures of GFAP did not increase (a small decrease was in fact found). Actin levels were unchanged and Fluoro-Jade staining found no indication of degenerating neurons in slice cultures exposed to three RDX blasts, suggesting that synaptic alterations can occur in the absence of cellular degeneration and gliosis. Together, these results indicate that detonated RDX explosives cause distinct losses of synaptic proteins before cell death, perhaps explaining the cognitive deficits in those blast-induced TBIs with no detectable neuropathology.

PROTEIN ACCUMULATION DISEASES

Dr. Bahr's research also aims to understand how age-related protein accumulation stress can be reduced for therapeutic purposes. Distinct protein accumulation events are suspected to lead to Alzheimer's, Parkinson's, Huntington's, ALS, and other diseases. The new confocal microscopy system assisted the following study being prepared for publication:

Accumulating protein species can lead to the activation of proteasomal and lysosomal pathways. However, many studies have indicated that the two pathways exhibit stress during Alzheimer-type protein accumulation events. In particular, the Aβ42 peptide has been shown to influence proteasomes and overall proteostasis. Here, low concentration Aβ42 applied to rat hippocampal slice cultures was found to reduce proteasome activity in correspondence with increased tau phosphorylation, as well as cause a significant loss of synaptophysin, a sensitive marker of synaptic integrity. When the slice cultures were treated with the proteasome inhibitor lactacystin, the nearly complete and rapid reduction in proteasome activity was not associated with lysosomal compromise, but rather with a >50% increase in activity of the lysosomal enzyme cathepsin B (CatB). Interestingly, the potential compensatory CatB response increased further over additional days of lactacystin treatment. To further assess this apparent inverse relationship between the proteasomal and lysosomal pathways, we tested whether enhancing the active form of CatB leads to proteasomal attenuation. Using the CatB-enhancing agent Z-Phe-Ala-diazomethylketone (PADK), an inverse effect was not found, but rather what appears to be an opposite effect, i.e. a tendency to increase proteasome activity. Surprisingly, in hippocampal slices with Aβ42-mediated proteasomal compromise, PADK indeed increased the proteasome activity to levels comparable to those found in control slices. Furthermore, PADK also reduced Aβ42-mediated tau phosphorylation, an event recently implicated as a consequence of changes in protein clearance efficiency. Such efficiency may involve cross-talk between proteasomes and lysosomes. These results suggest a distinct interaction between proteasomal and lysosomal systems, and they point to potential dual modulation against protein accumulation pathology linked to Alzheimer's disease and other dementias. New activity probes are currently being tested to assess modulation of protein clearance pathways in living brain tissue cultures.

INVASIVE FIRE ANTS

Professor Lisa Kelly of UNC Pembroke has been trained on the new confocal system. Dr. Kelly's research interest in the trophic ecology of the invasive fire ant has begun to benefit from the wide field view and long working distances of a confocal imaging system. The macro-observations of individual ants have already achieved images never seen with such resolution of ant body morphology. This will allow for diagnostic identification and may allow the capture of subtle differences in morphology of monogyne and polygyne fire ants --- differences that may be unapparent using conventional stereoscopic microscopes. The confocal system has provided a unique opportunity for undergraduate student experience and training.

NEMATODE INTERACTIONS

Professor Len Holmes of UNC Pembroke and his staff are being trained on the new confocal system. His research interest includes the interaction of a nematode species (*H. bacteriophora*) with its bacterial symbiotic partner (*P. luminescens*). The addition of the confocal microscope system will greatly facilitate Professor Holmes' work on the nematode-bacteria interactions, using novel fluorescent probes and image analysis.

NEUROMUSCULAR JUNCTION MODEL

Professor Robert Poage of UNC Pembroke is being trained on the new confocal system. Dr. Poage's research interest involves the dynamics of structural networks that modulate the neuromuscular junction and its functionality. This neuromuscular junction model, which uses frogs and rodents, has been among the most widely studied synaptic models. Dr. Poage's work manipulates ultrastructural features of the neuromuscular junction using neurotoxins, genetic regulation, or ionic manipulations. With the new microscope system, the Poage Lab will be able to probe important questions regarding the presynaptic calcium channel localization.

RESEARCH TRAINING CAPACITY

The new equipment has strengthened the research training capacity of UNCP. UNCP is a minority serving institution with 16% of the students being Native American, 32% are African American, and 4% are Hispanic/Latino. Faculty are dedicated to meeting the academic needs of minority students including mentoring undergraduate students in research and advanced technologies like confocal microscopy. Dr. Bahr's group has exposed many students to confocal techniques performed with the confocal imaging system, including undergraduates, a few Master students, as well as visiting and shadowing high school students. With the new system, UNCP students are engage in cutting edge research and are able acquire the skills and experience to prepare them for graduate programs and research careers in DOD-relevant areas. In addition, the STEM faculty of UNCP are committed to working with local K-12 students and teachers and local community college students. These students are regularly invited to tour and work in the Bahr Lab and other STEM labs on campus.

4. Bibliography (including previous studies that used confocal imaging)

Bahr BA, Wisniewski ML, and Butler D (2012) Positive lysosomal modulation as a unique strategy to treat age-related protein accumulation diseases. *Rejuvenation Res* 15:189-197.

Bendiske J, Caba E, Brown QB, and Bahr BA (2002) Intracellular deposition, microtubule destabilization, and transport failure: An 'early' pathogenic cascade leading to synaptic decline. J Neuropathol Exp Neurol 61:640-650.

Butler D, Brown QB, Chin D, Batey L, Karim S, Mutneja MS, Karanian DA, and Bahr BA (2005) Cellular responses to protein accumulation involve autophagy and lysosomal enzyme activation. Rejuvenation Res 8:227-237.

Butler D, Hwang J, Estick C, Nishiyama A, Kumar SS, Baveghems C, Young-Oxendine HB, Wisniewski ML, Charalambides A, and Bahr BA (2011) Protective effects of positive lysosomal modulation in Alzheimer's disease transgenic mouse models. *PLoS One* 6: e20501.

Carrasco DI, Bahr BA, Seburn KL, and Pinter MJ (2016) Abnormal response of distal Schwann cells to denervation in a mouse model of motor neuron disease. *Exp Neurol* 278:116-126.

Farizatto KL, Ikonne US, Ferrari MF, and Bahr BA (2016) Interaction between proteasomal and lysosomal systems influences Aβ effects in hippocampus. In preparation.

Filipovic R, Kumar SS, Bahr BA, and Loturco J (2014) Slice culture method for studying migration of neuronal progenitor cells derived from human embryonic stem cells (hESC). *Curr Protoc Stem Cell Biol* 29:1H.7.1-1H.7.14.

Hoffmann DB, Williams SK, Bojcevski J, Müller A, Stadelmann C, Naidoo V, Bahr BA, Diem R, and Fairless R (2013) Calcium influx and calpain activation mediate preclinical retinal neurodegeneration in autoimmune optic neuritis. *J Neuropathol Exp Neurol* 72:745-757.

Maltecca F, Baseggio E, Consolato F, Mazza D, Podini P, Young SM Jr, Drago I, Bahr BA, Puliti A, Codazzi F, Quattrini A, and Casari G (2015) Purkinje neuron Ca^{2+} influx reduction rescues ataxia in the spinocerebellar ataxia type 28 (SCA28) model. *J Clin Invest* 125:263-274.

Melo CV, Okumoto S, Gomes JR, Baptista MS, Bahr BA, Frommer WB, and Duarte CB (2013) Spatiotemporal resolution of BDNF neuroprotection against glutamate excitotoxicity in cultured hippocampal neurons. *Neuroscience* 237:66-86.

Munirathinam S and Bahr BA (2004). Repeated contact with subtoxic soman leads to synaptic vulnerability in hippocampus. *J Neuroscience Res* 77:739-746.

Smith M, Farizatto KL, Piehler T, Benjamin R, Almeida MF, and Bahr BA (2016) Blast waves from detonated RDX explosive lead to synaptic protein loss in hippocampal slice cultures.

Wisniewski ML, Hwang J, and Bahr BA (2011) Submicromolar Aβ42 reduces hippocampal glutamate receptors and presynaptic markers in an aggregation-dependent manner. *Biochim Biophys Acta* (*Mol. Basis of Disease*) 1812:1664-1674.